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Pharmacognostic standardization and HPTLC analysis of the leaves of *Hiptage benghalensis* (L.) Kurz

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ABSTRACT

Objective: To analyze the anatomical features, physiochemical characteristics and to carry out a high performance thin layer chromatography finger printing on the leaves of *Hiptage benghalensis*.
Methods: Anatomical characterization (organoleptic, microscopic and macroscopic analysis) and physico-chemical analysis (ash content, moisture content, foreign matter, extractive values, and fluorescence analysis) were carried out as per World Health Organization guidelines followed by high performance thin layer chromatography studies.
Results: Pharmacognostic studies revealed that leaf of *Hiptage benghalensis* had a wide and thick semi-circular midrib, thick and dorsiventral lamina, thick adaxial and narrow abaxial epidermis. They also had six to seven layered spongy parenchymal cells.
Conclusions: Results of the present study can serve as a marker for authentic identification and authentication of the plant of this taxon.

1. Introduction

Medicinal plants are natural resources, leading to valuable herbal products, which are often used in the treatment of various diseases. World Health Organization stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/or chemical markers and the fingerprint profiles[1]. Methods of standardization should consider all aspects that contribute to the quality of the herbal drugs like correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation and quantitative evaluation (ash values, extractive values[2]). The family Malpighiaceae consists of about 60 genera and 1 200 species of climbers, shrubs or treelets from native to tropical regions and is well developed in South America. They are known to contain tannins, proanthocyanins, and indole alkaloids. The Malpighiaceae is the most archaic family of the Polygalales[3]. *Hiptage benghalensis* (L.) Kurz (*H. benghalensis*) belongs to the family of Malpighiaceae and can also be found almost throughout India and north of South America. The bark, leaves and flowers of *H. benghalensis* are aromatic. They are useful in conditions of burning sensation, wounds, ulcers, inflammations, leprosy, scabies, cough, antihepatotoxic and rheumatism[4]. In Burma, the leaves of *H. benghalensis* are used to treat skin diseases. In Indonesia, the bark is used to heal wounds. In India, *H. benghalensis* is used to treat cough, asthma, leprosy, and quench thirst.

The plant has tremendous therapeutic potential with every part of the plant being used medicinally. In view of its pharmacological importance in traditional and modern system of medicine, it is necessary to develop a quality standard for this plant. Hence, the present investigation is an attempt to document the organoleptic features, quantitative values such as ash content, moisture content, extractive values and chromatographic profile of *H. benghalensis* that would serve as important parameters in assessing the quality of the sample for drug development.

2. Materials and methods

2.1. Collection and authentication of plant materials

Aerial parts of the selected plant were collected from the Eastern Ghats, Tamilnadu. The taxonomic identification and authentication of the plant was certified (PARC/2012/1238) by Prof. Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai.

2.2. Macroscopic analysis

Macroscopic features of the plant were analyzed by standard method[5]. The paraffin-embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was

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10–12 μm. Dewaxing of the sections was carried out by customary procedure. The sections were stained with toluidine blue[6]. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit.

2.3. Physiochemical analysis

The physiochemical parameters (ash and moisture content, foreign matter, extractive value) were determined[7]. Fluorescence of the drug was observed under day and UV light (254 nm) using various solvent extracts as well as treating with acids and alkaline solutions of the drug. The powder was treated with neutral solvents like hexane, benzene, chloroform, ethyl acetate, alcohol, acetone and acids like 1 mol/L hydrochloric acid, 50% sulphuric acid and alkaline solutions like aqueous and alcoholic 1 mol/L NaOH[8].

2.4. High performance thin layer chromatography (HPTLC)

HPTLC was performed on aluminium packed silica gel 60 F254 HPTLC plates (Merck). The selective mobile phase was poured into the chamber and left to equilibrate for 30 min. Samples were applied to the plates as sharp bands by means of CAMAG Linomat 5 samples applicator. After drying the spots in a current of air, the plates were placed in one trough of CAMAG twin trough chamber. The plate was then developed in an ascending one-dimensional mode in a saturated glass chamber. After separating, the plates were dried and the chromatographic bands were detected after spraying the plates with the suitable reagents. The plates were observed for various spots and R_f was recorded.

3. Results

3.1. Macroscopic and microscopic analysis

H. benghalensis belongs to the family of Malpighiaceae and is common in the foot hills. It is semi-evergreen, densely foliaceous liana on the forest trees. The leaves of the plant are ovate, elliptic oblong, with the length of 7–9 cm and 4–6 cm broad. The lamina is coriaceous, glaucous below, glabrous above. There are two glands at the base of the leaf. The leaf margins are entire. Petiole is 1 cm long. It contains five sepals that are persistent, longer than the petals. Flowers appear with five petals which are white with one of its petal appearing with pink and yellow blotch.

There were ten stamens with three unequal ovaries celled with one ovule in each cell. The leaf consisted of wide and thick semi-circular midrib and thick lamina (Figure 1). The midrib was composed of unistratose epidermal layer of squarish thick walled cells and some of the epidermal cells bearing non-grandular trichomes (Figure 2).

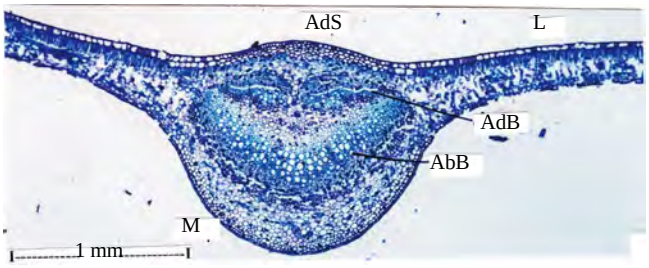

Figure 1. Transverse section of leaf through midrib.
AdS: Adaxial side; AdB: Adaxial bundle; AbB: Abaxial bundle; L: Lamina; M: Midrib.

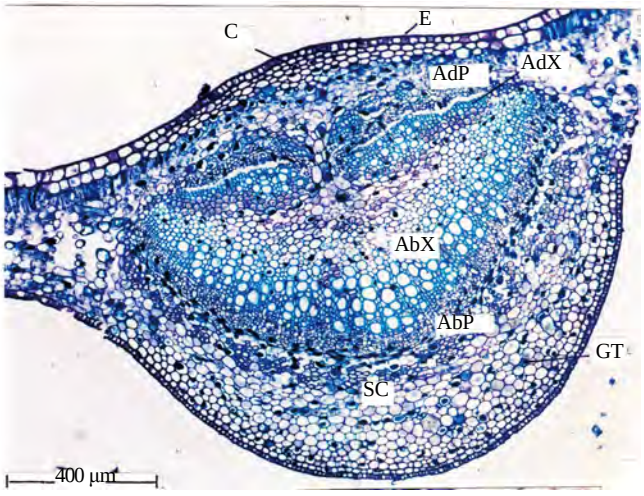

Figure 2. Transverse section of midrib enlarged.
C: Collenchyma; AdP: Adaxial phloem; AbP: Abaxial phloem; AbX: Abaxial xylem; AdX: Adaxial xylem; E: Epidermis; GT: Ground tissue; SC: Subsidiary cells.

The plano-convex vascular strand was ensheathed with thick layer of sclerenchyma cells. The lamina was dorsiventral. The adaxial epidermis was thick with rectangular or squarish cells. The abaxial epidermis was narrow and cylindrical and the cuticle was also thick. The palisade cells were long, cylindrical and were arranged with gaps in between (Figure 3). The internal structure of the lamina was similar to that of the middle portion which was composed of adaxial zone of cylindrical palisade cells and abaxial loosely arranged spherical spongy parenchyma cells (Figure 4).

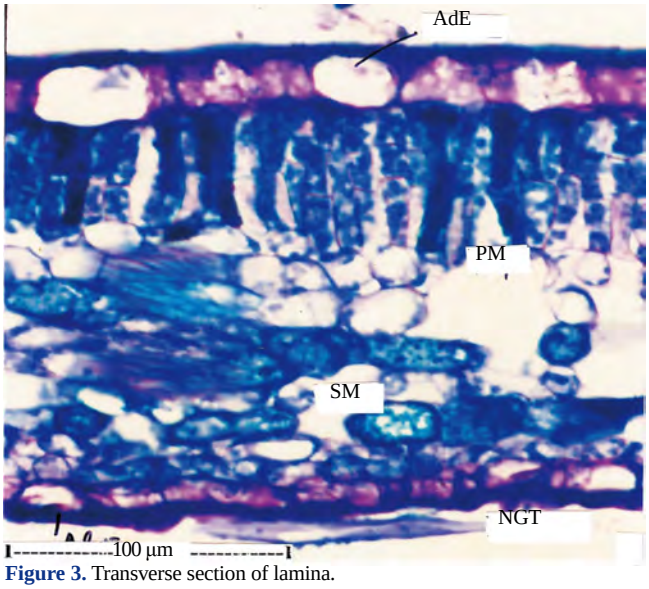

Figure 3. Transverse section of lamina.
AdE: Adaxial epidermis; SM: Spongy mesophyll; PM: Palisade mesophyll; NGT: Non-glandular trichome.

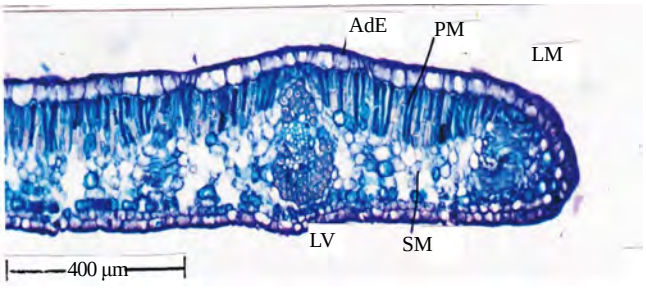

Figure 4. Transverse section of leaf margin.
AdE: Adaxial epidermis; LM: Leaf margin; LV: Lateral vein; SM: Spongy mesophyll; PM: Palisade mesophyll.

The stomata were paracytic type. Each guard cell had lateral, parallel subsidiary cells of equal size and shape (Figure 5). The petiole consisted of a thin epidermal layer of darkly stained epidermal cells. The ground tissue was homogenous, parenchymatous, comprising circular compact cells. Many cells possessed tannin content. The vascular system consisted of a deep wide bowl shaped lower part and a horizontal flat adaxial plate (Figure 6).

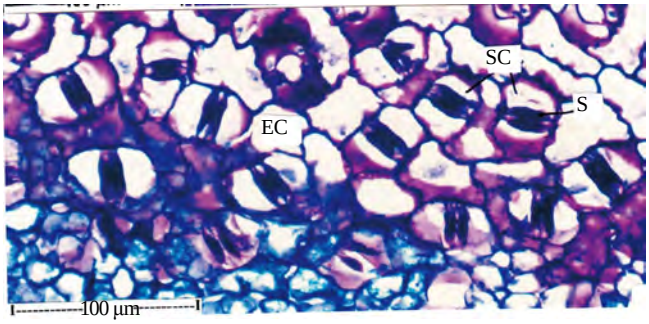

Figure 5. Paradermal section of abaxial epidermis showing stomata.
EC: Epidermal cell; S: Stomata; SC: Subsidiary cells.

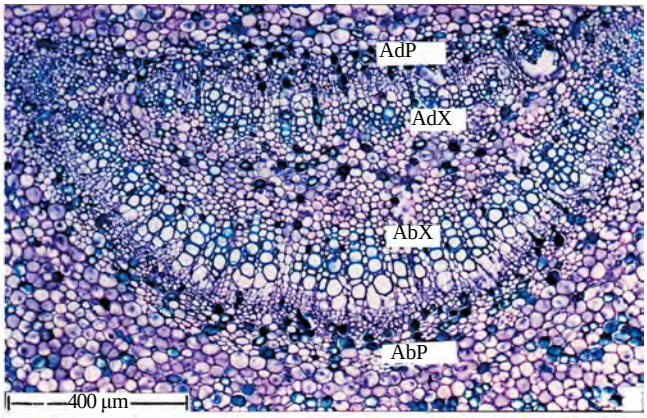

Figure 6. The vascular strand of the petiole enlarged.
AdP: Adaxial phloem; AbP: Abaxial phloem; AbX: Abaxial xylem; AdX: Adaxial xylem.

3.4. Powder microscopic results

Fragments of adaxial epidermal peeling were frequently seen in the powder (Figure 7). The epidermis lacked stomata and the abaxial epidermis bore dense stomata. The guard cells were laterally stretched with horizontally elongated stomatal pores. The subsidiary cells had prominent echinate out growths.

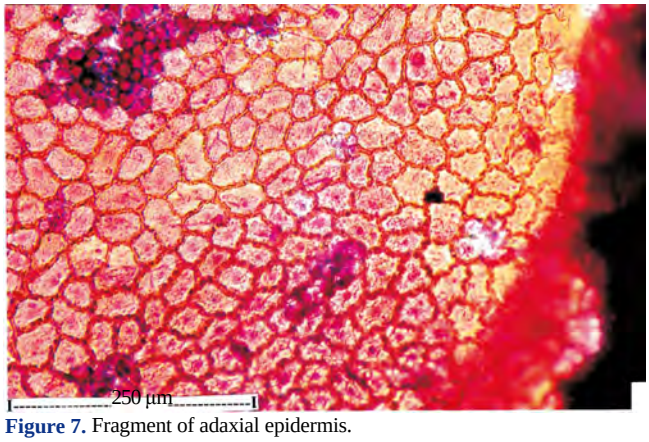

Figure 7. Fragment of adaxial epidermis.

3.5. Physiochemical analysis

Physiochemical characters like ash and moisture content, extractive values and foreign matters were determined. The plant sample showed a higher total ash content (6.3%) than acid soluble (1.3%) and water-soluble ash (4.5%). Foreign matter was negligible (2.3%). The plant sample showed the highest extractive value in alcohol (11.02%) followed by water (6.14%), chloroform (2.32%), hexane (1.95%) and ethyl acetate (1.15%). Fluorescence analysis of the sample under UV and daylight were studied and the results were tabulated in Table 1.

Table 1

Fluorescence analysis of *H. benghalensis* drug powder.

Treatment	Daylight		UV light	
	24 h	48 h	24 h	48 h
Chloroform	Light yellow	Green	Brown	Black
Hexane	Yellow	Yellow	Green	Green
Alcohol	Dark green	Dark green	Green	Dark green
Benzene	Greenish yellow	Green	Green	Green
Acetone	Green	Dark green	Dark green	Dark green
Ethyl acetate	Light brown	Brown	Light green	Green
Water	Dark green	Dark green	Dark green	Dark green

3.6. HPTLC

HPTLC profiles of the ethanol extract of *H. benghalensis* on silica gel 60F254 HPTLC plate using toluene: ethyl acetate (93:7) as mobile phase were given in Figure 8. Thin layer chromatography profiles revealed 4 spots under UV (254 nm) and 8 spots under UV (366 nm) (Table 2). The R_f values and the peak area percentage were observed and given in Table 3 and their chromatogram fingerprint was shown in Figures 9 and 10.

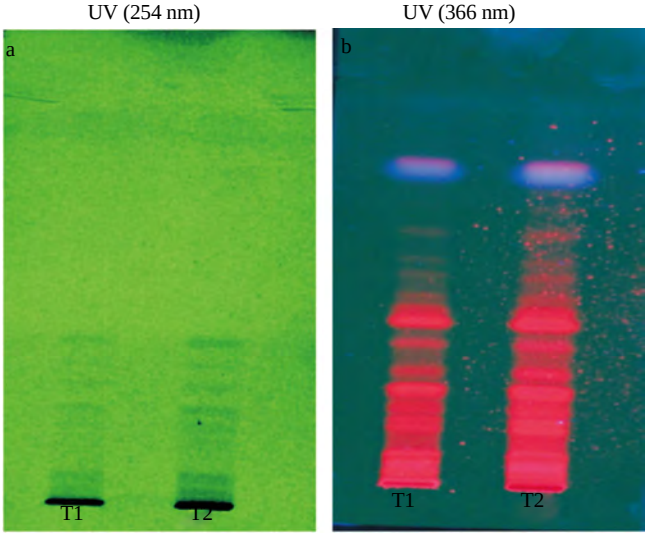

Figure 8. Thin layer chromatographic profiles of ethanolic extract.

Table 2

Thin layer chromatographic profiles of ethanolic extract.

R_f value	Colour of the spot	
	UV (254 nm)	UV (366 nm)
0.22	Green	Red
0.29	Green	Red
0.36	Green	Red
0.45	Green	Red
0.59	-	Red
0.61	-	Red
0.80	-	Red
0.92	-	Blue

Table 3
HPTLC finger print of ethanol extract (10 μL and 20 μL).

	R_f (10 μL)						R_f (20 μL)							
	0.11	0.17	0.22	0.29	0.36	0.80	0.22	0.29	0.36	0.45	0.59	0.61	0.80	0.92
Peak area (%)	12.25	26.74	3.96	6.13	28.88	7.87	4.92	6.51	26.16	2.81	0.88	1.46	7.34	1.15

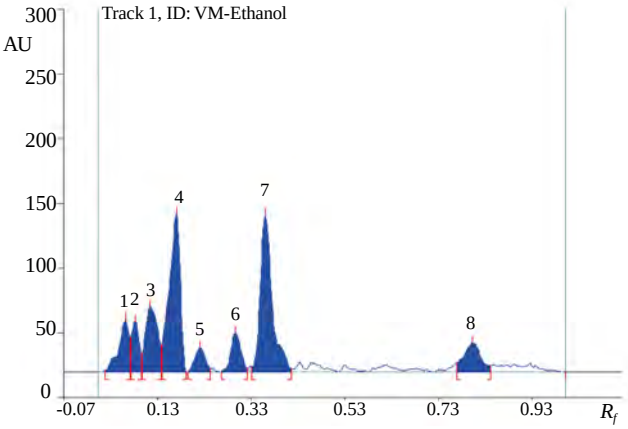

Figure 9. HPTLC finger print of ethanol extract of *H. benghalensis* (10 μL).

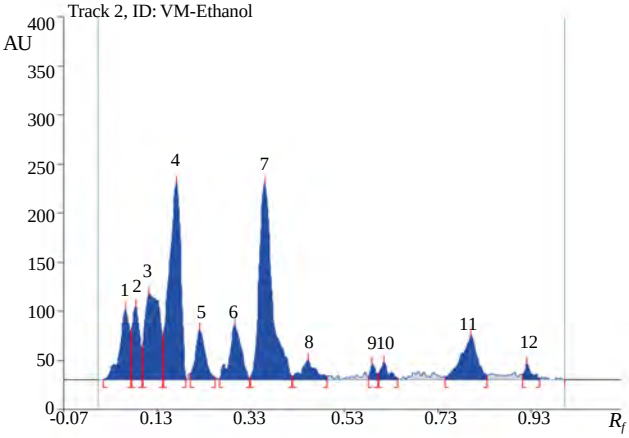

Figure 10. HPTLC finger print of ethanol extract of *H. benghalensis* (20 μL).

4. Discussion

Organoleptic study of the leaf powder of *H. benghalensis* revealed that it was green in colour and had an aromatic odour and a bitter taste. Macroscopic and microscopic results showed that the leaf consisted of a wide and thick semi-circular midrib, thick lamina. The lamina was dorsiventral. The adaxial epidermis was thick with rectangular or squarish cells and cuticle. The abaxial epidermis was narrow and cylindrical. Epidermal trichomes were frequently seen on the epidermal cells. Spongy parenchymal cells were spherical or cylindrical and were loosely arranged. They had 6 or 7 layers.

The vascular strands consisted of thin-walled xylem elements and thin layer of phloem on the outer border. Previous pharmacognostic studies on this plant[4] have also documented similar results.

The physico-chemical parameters such as ash content, moisture content, extractive values and determination of foreign matters play an important role in evaluating the quality and purity of the plant drug. Determination of florescence is a sensitive and accurate technique, and the fluorescence colour is specific for each compound[9]. HPTLC technique finds the maximum use in pharmaceutical industries for process development, identification and detection of adulterants in herbal products and in quality control of herbs and health foods[10]. The HPTLC results are in accordance with previously documented phytochemical investigations on the plant which also revealed the presence of terpenoids[11].

Correct identification and quality assurance is an essential prerequisite and contributes to the safety and efficacy of herbal medicine and the chromatographic profiling of a plant is globally accepted to establish variability within the same herbal material. The present investigation has been an attempt to document the anatomical characteristics and HPTLC profiling of *H. benghalensis* that can serve as an important therapeutic and diagnostic tool to evaluate herbal medicine as indigenous resources.

Conflict of interest statement

We declare that we have no conflict of interest.

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